

WHAT IS CLAIMED IS:

*Sub A1*

1. A reverse transcriptase which has been modified or mutated to increase or enhance thermostability.
2. The reverse transcriptase of claim 1, wherein the reverse transcriptase has one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:
  - (a) leucine 52 of M-MLV reverse transcriptase;
  - (b) tyrosine 64 of M-MLV reverse transcriptase;
  - (c) lysine 152 of M-MLV reverse transcriptase;
  - (d) histidine 204 of M-MLV reverse transcriptase;
  - (e) methionine 289 of M-MLV reverse transcriptase; and
  - (f) threonine 306 of M-MLV reverse transcriptase.
3. The reverse transcriptase of claim 2, which is M-MLV reverse transcriptase.
4. The reverse transcriptase of claim 3, wherein leucine 52 is replaced with proline.
5. The reverse transcriptase of claim 3, wherein tyrosine 64 is replaced with arginine.
6. The reverse transcriptase of claim 3, wherein lysine 152 is replaced with methionine.
7. The reverse transcriptase of claim 3, wherein histidine 204 is replaced with arginine.

8. The reverse transcriptase of claim 3, wherein methionine 289 is replaced with leucine.

9. The reverse transcriptase of claim 3, wherein threonine 306 is replaced with either lysine or arginine.

10. The reverse transcriptase of claim 3, wherein the reverse transcriptase has a mutation or modification at amino acids histidine 204 and threonine 306.

11. The reverse transcriptase of claim 10, wherein histidine 204 is replaced with arginine and threonine 306 is replaced with either lysine or arginine.

12. The reverse transcriptase of claim 1, which retains at least 50% of reverse transcriptase activity after heating to 50°C for 5 minutes.

13. The reverse transcriptase of claim 1, which retains at least 70% of reverse transcriptase activity after heating to 50°C for 5 minutes.

14. The reverse transcriptase of claim 1, which retains at least 85% of reverse transcriptase activity after heating to 50°C for 5 minutes.

15. The reverse transcriptase of claim 1, which retains at least 95% of reverse transcriptase activity after heating to 50°C for 5 minutes.

16. The reverse transcriptase of claim 1, wherein the reverse transcriptase has one or more properties selected from the group consisting of:

- (a) reduced or substantially reduced RNase H activity;
- (b) reduced or substantially reduced terminal deoxynucleotidyl transferase activity; and
- (c) increased fidelity.

17. The reverse transcriptase of claim 16, wherein the reverse transcriptase has reduced or substantially reduced RNase H activity.

18. The reverse transcriptase of claim 16, wherein the reverse transcriptase has reduced or substantially reduced terminal deoxynucleotidyl transferase activity.

19. The reverse transcriptase of claim 18, wherein the reverse transcriptase has one or more one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) tyrosine 133 of M-MLV reverse transcriptase;
- (b) threonine 197 of M-MLV reverse transcriptase; and
- (c) phenylalanine 309 of M-MLV reverse transcriptase.

20. The reverse transcriptase of claim 19, which is M-MLV reverse transcriptase.

21. The reverse transcriptase of claim 20, wherein tyrosine 133 is replaced with alanine.

22. The reverse transcriptase of claim 20, wherein threonine 197 is replaced with glutamic acid.

23. The reverse transcriptase of claim 20, wherein phenylalanine 309 is replaced with asparagine.

24. The reverse transcriptase of claim 16, wherein the reverse transcriptase has increased fidelity.

25. The reverse transcriptase of claim 24, wherein the reverse transcriptase has one or more one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) tyrosine 64 of M-MLV reverse transcriptase;
- (b) arginine 116 of M-MLV reverse transcriptase; and
- (c) glutamine 190 of M-MLV reverse transcriptase; and
- (d) valine 223 of M-MLV reverse transcriptase.

26. The reverse transcriptase of claim 1, wherein the reverse transcriptase is selected from the group consisting of M-MLV, RSV, AMV, and HIV reverse transcriptases.

27. The reverse transcriptase of claim 26, wherein the reverse transcriptase is selected from the group consisting of M-MLV RNase H- reverse transcriptase, RSV RNase H- reverse transcriptase, AMV RNase H- reverse transcriptase, RAV RNase H- reverse transcriptase, and HIV RNase H- reverse transcriptase.

28. The reverse transcriptase of claim 26, wherein the reverse transcriptase is an M-MLV reverse transcriptase.

29. The reverse transcriptase of claim 28, wherein aspartic acid 524 is replaced with glycine, glutamic acid 562 is replaced with glutamine, and aspartic acid 583 is replaced with asparagine.

30. A vector comprising nucleic acid encoding the reverse transcriptase of claim 1.

31. The vector of claim 30, wherein the nucleic acid is operably linked to a promoter.

32. The vector of claim 31, wherein the promoter is selected from the group consisting of a  $\lambda$ -P<sub>L</sub> promoter, a *tac* promoter, a *trp* promoter, an ara BAD promoter and a *trc* promoter.

33. A host cell comprising the vector of claim 30.

34. A method of producing a reverse transcriptase, the method comprising:

- (a) culturing the host cell of claim 33;
- (b) expressing the nucleic acid; and
- (c) isolating the reverse transcriptase from the host cell.

35. The method of claim 34, wherein the host cell is an *Escherichia coli*.

36. A method for reverse transcription of one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with one or more reverse transcriptases of claim 1; and
- (b) incubating the mixture of (a) under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of the one or more templates.

37. The method of claim 36, wherein the nucleic acid template is a messenger RNA molecule or a population of mRNA molecules.

38. The method of claim 37, the method further comprising the step of incubating the one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of the one or more first nucleic acid molecules.

39. A cDNA molecule made according to the method of claim 36.

40. A cDNA molecule made according to the method of claim 38.

41. A method for amplifying one or more nucleic acid molecules, the method comprising:

(a) mixing one or more nucleic acid templates with one or more reverse transcriptases of claim 1 and one or more DNA polymerases; and

(b) incubating the mixture of (a) under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of the one or more templates.

42. A method for sequencing one or more nucleic acid molecules, the method comprising:

(a) mixing one or more nucleic acid molecules to be sequenced with one or more primers, one or more reverse transcriptases of claim 1, one or more nucleotides and one or more terminating agents;

(b) incubating the mixture of (a) under conditions sufficient to synthesize a population of molecules complementary to all or a portion of the one or more molecules to be sequenced; and

(c) separating the population to determine the nucleotide sequence of all or a portion of the one or more molecules to be sequenced.

43. A method for sequencing a nucleic acid molecule, the method comprising:

(a) mixing a nucleic acid molecules to be sequenced with one or more primers, one or more reverse transcriptases of claim 1, one or more nucleotides and one or more terminating agents;

(b) incubating the mixture of (a) under conditions sufficient to synthesize a population of molecules complementary to all or a portion of the molecule to be sequenced; and

(c) separating members of the population to determine the nucleotide sequence of all or a portion of the molecule to be sequenced.

44. A kit for use in reverse transcription, amplification or sequencing of a nucleic acid molecule, the kit comprising one or more reverse transcriptases of claim 1.

45. The kit of claim 44, the kit further comprising one or more components selected from the group consisting of one or more nucleotides, one or more DNA polymerases, a suitable buffer, one or more primers and one or more terminating agents.

46. The kit of claim 45, wherein the terminating agent is a dideoxynucleotide.

47. The kit of claim 44, wherein the reverse transcriptase is an M-MLV reverse transcriptase.

48. The kit of claim 47, wherein the reverse transcriptase has one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) leucine 52 of M-MLV reverse transcriptase;
- (b) tyrosine 64 of M-MLV reverse transcriptase;
- (c) lysine 152 of M-MLV reverse transcriptase;
- (d) arginine 204 of M-MLV reverse transcriptase;
- (e) methionine 289 of M-MLV reverse transcriptase; and
- (f) threonine 306 of M-MLV reverse transcriptase.

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